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Differential expression of microRNAs and their messengerRNA targets in men with normal spermatogenesis versus Sertoli cell-only syndrome

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ABSTRACT

Objective: To identify the profiling of testicular microRNAs (miRNAs) that are differentially expressed in men with normal spermatogenesis versus Sertoli cell-only syndrome (SCOS), to predict the miRNA target genes, and to determine the molecular networks/pathways constituted by miRNA targets.
Materials and methods: Nine obstructive azoospermic men with normal spermatogenesis and nine SCOS men were enrolled in this study. Testicular tissues from three men with normal spermatogenesis and three men with SCOS were pooled respectively for miRNA microarray analysis, and the other 12 testicular specimens were used for subsequent quantitative real-time polymerase chain reaction analysis for validation. Moreover, the predicted targets of the differentially expressed miRNAs were identified *in silico* and uploaded to Ingenuity Pathway Analysis for further network/pathway analysis.
Results: Three miRNAs that were upregulated (miR-136, miR-630, and miR-663) and seven miRNAs that were downregulated (miR-15b, miR-18a, miR-25, miR-30a-5p, miR-34b, miR-93, and miR-126) in SCOS specimens were identified. A total of 51 spermatogenesis-related targets were predicted *in silico*. Results from quantitative reverse transcription polymerase chain reaction generally correlate with microarray and computational analysis. Three significant molecular networks and five canonical pathways associated with biological functions of steroidogenesis and spermatogenesis were identified.
Conclusions: Aberrant expression of 10 differentially expressed miRNAs might result in dysregulation of steroidogenesis and spermatogenesis that may ultimately lead to SCOS.

1. Introduction

Sertoli cell-only syndrome (SCOS), also known as germ cell aplasia, is a testicular phenotype that was first described in 1947 by Del Castillo et al.[1] The diagnosis of SCOS is based on the testicular histopathology that exhibits a complete absence of germ cells with only Sertoli cells lining the seminiferous tubules, reduced seminiferous tubule diameter, and thickened seminiferous tubule walls. Clinically, SCOS patients present with azoospermia, normal virilization, and normal to atrophic testis. Although testicular maldescent, radiotherapy, chemotherapy, and genetic abnormalities have been linked to the SCOS phenotype,[2–5] the causes of SCOS at the molecular level remain largely unknown. Complementary DNA microarray experiments were previously used to profile gene expression in the testes of infertile men, and unique gene expression signatures for SCOS have been reported.[6,7] However, little is known about the mechanisms involved in the regulation of gene expression in SCOS testes.

MicroRNAs (miRNAs) ranging between 18 bp and 25 bp are highly conserved, small, noncoding RNAs that repress expression of target genes through messenger RNA (mRNA) cleavage, translation repression, or chromatin modification, and silencing.[8–11] It is estimated that approximately 500–1000 miRNAs are encoded in the human genome and up to 30% of human protein-coding genes may be miRNA targets.[8,12] It has been shown that miRNAs have important roles in diverse biological processes, including cellular development, cell proliferation, cell differentiation, apoptosis, organogenesis, fat metabolism, hematopoiesis, and cancer

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formation. The expressions of miRNAs are considered to be tissue- and developmental stage-specific.[8]

To date, there are increasing evidences showing that miRNAs may play crucial roles in mammalian spermatogenesis. Numerous miRNAs preferentially or exclusively expressed in mouse testes have been identified.[13,14] Mirn122a inhibits *Tnp2* mRNA translation *in vitro* by degrading the mRNA. *Tnp2* is a testis-specific gene which is required in chromatin remodeling during spermiogenesis.[15] Chromatoid body known as a perinuclear granule in spermatocytes and spermatids expresses components of miRNA machinery, indicating that miRNAs play a role in late spermatogenesis.[16] *Dicer1* is required for all miRNA processing, and lack of *Dicer1* specifically in germlines resulted in spermatogenic defects in multiple stages of germ cell differentiation.[17] miR-17-92 and miR-290-295 clusters that have been shown to promote cell cycling are highly expressed in primordial germ cell and spermatogonia, indicating that miRNA biogenesis is also required for the proliferation or early differentiation of stem cell population.[18] However, all the above-mentioned studies were performed in mice; little is known about the expressions and biological functions of miRNAs in human testis.

Spermatogenesis relies heavily on post-transcriptional gene regulation, and defects in the transcriptional regulation could profoundly influence germ cell proliferation and differentiation, leading to germ cell apoptosis and male infertility.[19–21] Given that miRNAs are recognized to modulate post-transcriptional regulation of target mRNA, we hypothesize that aberrant expression of miRNAs may be involved in human spermatogenic failure. This study was conducted to identify the testicular miRNAs that are differentially expressed between infertile men with normal spermatogenesis and SCOS, to predict their spermatogenesis-related target genes, and to explore the molecular networks/pathways constituted by miRNA targets.

2. Materials and methods

2.1. Patients and testicular specimens

A total of 18 azoospermic men (9 men with obstructive azoospermia and normal spermatogenesis and 9 men with SCOS) were enrolled in this study. The diagnosis of azoospermia was based on at least two separate semen analyses after centrifugation of semen samples. None of these 18 patients had a chromosomal abnormality or Y chromosome microdeletion. Patients with normal follicle-stimulating hormone levels, testicular volume, and histology in the testicular biopsy were categorized as having obstructive azoospermia and normal spermatogenesis. SCOS was defined by the lack of germ cells, with only Sertoli cells lining the seminiferous tubules in a testicular biopsy. These patients underwent testicular biopsy and/or testicular sperm extraction and agreed to provide a small piece of testicular tissue for the study. All SCOS patients enrolled were “complete” SCOS in whom failed testicular sperm extraction and no spermatogenesis in testicular biopsies were found. Testicular tissues from three men with normal spermatogenesis and three men with SCOS were pooled, respectively, for miRNA microarray analysis, and the other 12 testicular specimens were used for subsequent quantitative real-time polymerase chain reaction (qRT-PCR) for validation. This study was approved by the Institutional Review Board of our institute (2008005) and written informed consent was obtained from all participants.

2.2. Total RNA and miRNA isolation

Total RNA was extracted from testicular tissues homogenized in Trizol reagent (Invitrogen, Life Technologies, Carlsbad, CA, USA). Total RNA, including miRNA, was isolated using a miRNeasy Mini Kit (Qiagen, Inc., Valencia, CA, USA) according to the manufacturer's instruction. RNA concentration was assessed using the Nanodrop ND-1000 spectrophotometer (Nanodrop Technologies, Wilmington, DE, USA) and RNA quality was determined on an Agilent 2100 Bioanalyzer (Agilent Technologies, Palo Alto, CA, USA).

2.3. miRNA microarray analysis

For miRNA profiling, miRNA samples extracted from three normal spermatogenesis samples and three SCOS samples were pooled, respectively. RNA samples were tagged with T4 RNA ligase (GE healthcare, Chalfont Saint Giles, UK) by incubation at 16°C for 2 hours. The labeled samples were hybridized to Agilent human miRNA microarrays (version 2.0; Agilent Technologies), which contains probes for 534 human miRNAs based on the Sanger microRNA database version 8.2.[22] Hybridizations were carried out in duplicate. After hybridization and wash steps, the arrays were scanned using a laser confocal scanner (G2505B; Agilent Technologies) and the images were acquired with feature extraction software. The miRNA spot intensity values in scanned images were determined and background-adjusted intensities were normalized using a scatter plot. miRNAs that were differentially expressed between normal and SCOS specimens (fold change > 2, $p < 0.05$) were identified using GeneSpring 7.3.1 software (Agilent Technologies).

2.4. qRT-PCR analysis of miRNA expression

Four miRNAs were randomly selected for transcript level determination. Transcript levels of two upregulated (miR-136 and miR-630) and two downregulated (miR-25 and miR-126) miRNAs were determined using qRT-PCR in six normal and six SCOS individuals whose tissues were not used in the miRNA microarray analysis. Ten nanograms of total RNA were reverse transcribed using TaqMan MicroRNA Reverse Transcription Kit (Applied Biosystems, Foster City, CA, USA). Quantitative PCR was carried out using TaqMan Universal PCR Master Mix and a hsa-miR assay system on an ABI Prism 7900HT (Applied Biosystems). The reactions were incubated in a 96-well optical plate at 95°C for 10 minutes, followed by 40 cycles of 95°C for 15 seconds and 60° for 1 minute. The transcript level of each miRNA was normalized to the transcript level of U6 small nuclear RNA. The expression of miRNA relative to U6 small nuclear RNA was determined using the $2^{-\triangle\triangle Ct}$ method as described previously.[23]

2.5. miRNA spermatogenesis-related target prediction

The predicted targets of the differentially expressed miRNAs were identified *in silico*. Two bioinformatic algorithms, TargetScan[24] and PicTar,[25] were used for computational identification of putative miRNA targets. Given that miRNAs potentially regulate a large number of target transcripts, we further performed gene ontology (GO) analysis to identify the subset of predicted targets that are involved in spermatogenesis. Target genes were assigned functions based on the GO categories. Genes significantly associated with “spermatogenesis-related” GO categories, including gametogenesis, germ cell development, male gamete generation, spermatogenesis, spermatid development, spermatid differentiation, sperm chromatin condensation, and reproductive physiology process, were selected into our target list.

2.6. qRT-PCR analysis of target mRNA expression

Transcript levels of eight targets of miR-630 were determined using qRT-PCR in six normal and six SCOS individuals. Total RNA

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isolation and complementary DNA preparation from testicular tissue were performed as described previously.[26] The *HMBS* gene was used as an endogenous control. Primer sequences are available from the authors. The quantitative amplification was performed in an ABI Prism 7900HT Sequence Detection System (Applied Biosystems). A negative control without template was included in each set of qRT-PCR assays. The reactions were incubated in a 96-well optical plate at 95°C for 10 minutes, followed by 40 cycles of 95°C for 15 seconds and 60° for 1 minute. The expression of mRNA relative to *HMBS* was determined using the $2^{-\triangle\triangle Ct}$ method.

2.7. Biological function, network identification, and canonical pathway analysis of target genes

To explore the biological functions, potential molecular networks, and canonical pathways these spermatogenesis-related targets constitute, we uploaded these targets into Ingenuity Pathway Analysis (IPA; Ingenuity Systems, www.ingenuity.com). Each gene identifier was mapped to its corresponding gene object in the Ingenuity Pathways Knowledge Base. Fischer's exact test was used to calculate a *p* value determining the probability that each molecular and cellular function assigned to that data set is due to chance alone. These genes, called focus genes, were overlaid onto a global molecular network developed from information contained in the Ingenuity Pathways Knowledge Base. Networks of these focus genes were then algorithmically generated based on their connectivity.

Canonical pathways analysis identified the pathways from the IPA library of canonical pathways that were most significant to the data set. The significance of the association between the data set and the canonical pathway was measured in two ways: (1) a ratio of the number of genes from the data set that map to the pathway divided by the total number of genes that map to the canonical pathway is displayed; and (2) a *p* value calculated using Fischer's exact test determining the probability that the association between the genes in the dataset and the canonical pathway is explained by chance alone.

2.8. Statistical analysis

All qRT-PCR assays described above were performed in triplicate in at least three independent experiments. Data were expressed as means ± standard error of the mean. Unpaired *t*-test was performed to compare the transcript levels of miRNAs and mRNAs between normal and SCOS individuals. A p value < 0.05 was considered as significant.

3. Results

3.1. Differential expression of miRNAs between normal and SCOS testes

Of the 534 miRNAs, a total of 64 miRNAs exhibited differential expression with a fold change more than two-fold and a p value less than 0.05 between normal and SCOS specimens. Twenty-three miRNAs were upregulated (2–12.4-fold) and 41 miRNAs were downregulated (0.004–0.49-fold) in SCOS specimens. For increased confidence, the raw intensities of hybridization signals less than 100 were excluded. Therefore, three upregulated miRNAs (miR-136, miR-630, and miR-663) and seven downregulated miRNAs (miR-15b, miR-18a, miR-25, miR-30a-5p, miR-34b, miR-93, and miR-126) in SCOS specimens were identified (Figure 1A, Table 1).

3.2. Validation of miRNA expression

To validate the microarray results, transcript levels of two upregulated (miR-136 and miR-630) and two downregulated (miR-25 and miR-126) miRNAs were quantified using qRT-PCR analysis. In accordance with miRNA microarray results, the transcript levels of miR-25 and miR-126 were significantly decreased in the SCOS group (Figures 1B and 1C), and the transcript levels of miR-136 and miR-630 were significantly increased in the SCOS group (Figures 1D and 1E).

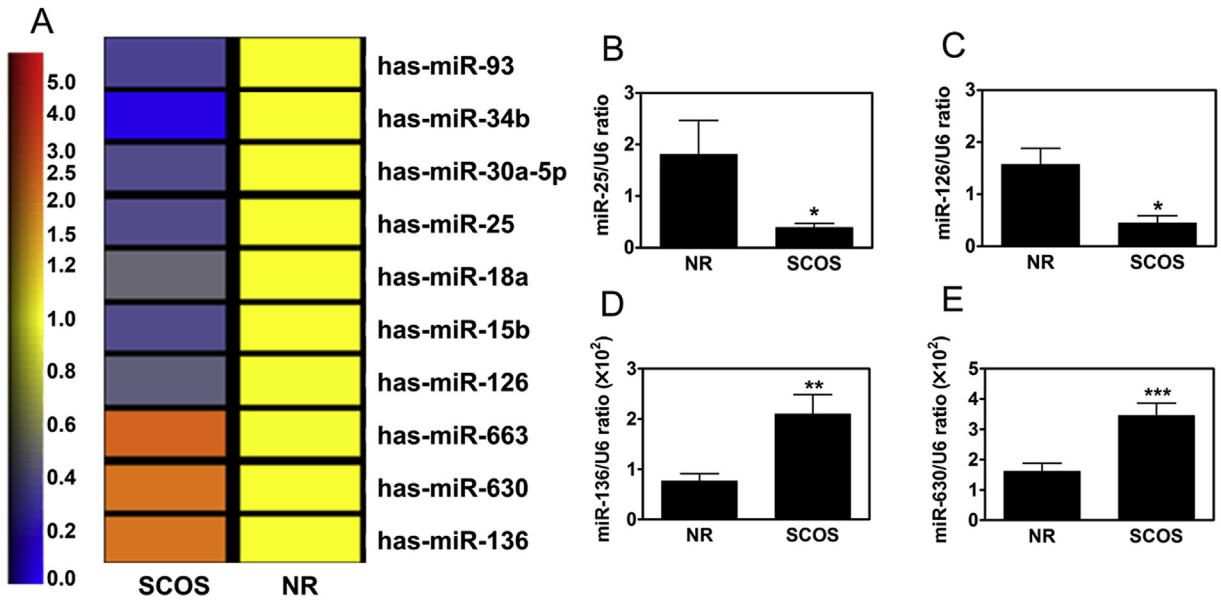

Figure 1. Profiles of microRNA (miRNA) expression. (A) The heat map represents clustering of the 10 significantly up- and downregulated miRNAs (fold change > 2, $p < 0.05$) in Sertoli cell-only syndrome (SCOS) testes compared with normal testes (NR); (B–E) in quantitative real-time polymerase chain reaction analysis, miR-25 and miR-126 transcripts were significantly decreased, whereas miR-136 and miR-630 transcripts were significantly increased in SCOS versus normal testes. * $p < 0.05$. ** $p < 0.01$. *** $p < 0.001$.

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Table 1
Differentially expressed microRNAs (miRNAs) between normal (NR) and Sertoli cell-only syndrome (SCOS) testes, their chromosomal locations, and spermatogenesis-related targets.

Human (hsa-) miRNA	Chromosome	Chromosomal location	Mean intensity in NR	Mean intensity in SCOS	SCOS/NR ratio	Spermatogenesis-related targets
hsa-miR-136	14q32.31	Intergenic	590	1321	2.238	*TNP2*; *H1FNT*; *ZSCAN2*; *SPATA19*
hsa-miR-630	15q24.1	Intergenic	573	1242	2.167	*HMGCR*; *SPAM1*; *SPAG1*; *CRISP3*; *PSME4*; *TAC3*; *GNAS*; *SOX30*
has-miR-663	20p11.1	Intergenic	590	1498	2.539	*FAM12A*; *AKAP3*; *FCGRT*; *APOE*; *ACHE*; *STARD3*; *MVD*
hsa-miR-15b	3q26.1	Intronic	4074	1579	0.387	*DUSP13*; *CHEK1*; *NME5*; *ODF1*; *PTCH2*; *PPAP2A*; *PROK2*
hsa-miR-18a	13q31.3	Intronic	738	362	0.491	*ADAM28*; *BCL2L10*; *INSL3*; *MAK*; *STRBP*; *PRM3*; *SLC22A16*; *PPAP2A*
hsa-miR-25	7q22.1	Intronic	1467	540	0.368	*MAST2*; *TESK1*; *ODF1*; *OAZ3*
hsa-miR-30a-5p	6q13	Intergenic	1946	771	0.396	Not available
hsa-miR-34b	11q23.1	Intergenic	9889	685	0.069	*BCL2L10*; *SERPINA5*; *INSL3*; *AZI1*; *SPAG11B*; *DZIP1L*; *SPATA4*; *PPAP2A*
hsa-miR-93	7q22.1	Intronic	925	323	0.349	*ABCB9*; *TCFL5*; *MAK*; *STRBP*; *DMC1*; *CCNA1*; *CASC5*
hsa-miR-126	9q34.3	Intronic	388	1776	0.457	*ADAM28*; *CRISP1*; *DDX25*; *ELL3*; *SPAG11B*; *PRKACG*

3.3. Prediction of miRNA targets

The predicted target genes for each miRNAs are shown in Table 1. A total of 51 targets were identified; however, significant spermatogenesis-related targets for miR-30a-5p were not available. IPA suggested that these target genes were significantly enriched for functional annotations associated with cellular development, cell cycle, cellular assembly and organization, cellular movement, and cell-to-cell signaling and interaction. The genes that are involved in each function according to IPA are shown in Table 2.

3.4. Determination of transcript levels of miR-630 targets

To validate the mRNA expressions of miRNA targets between normal and SCOS samples, miR-630 was randomly selected and the transcript levels of its target genes were determined using qRT-PCR (Figure 2). As shown in Figure 3, the transcript levels of *SPAG1*, *PSME4*, *TAC3*, and *SOX30* were significantly decreased in the SCOS group, whereas no significant differences were found in the *HMGCR*, *SPAM1*, *CRISP3*, and *GNAS* transcript levels between the normal and SCOS group.

Table 2
Molecular and cellular functions of 51 spermatogenesis-related target genes.

Function	No. of molecule	Molecule	*p*
Cellular development	24	*ABCB9*, *ACHE*, *ADAM28*, *AZI1*, *BCL2L10*, *CCNA1*, *CHEK1*, *DDX25*, *DMC1*, *DUSP13*, *H1FNT*, *INSL3*, *MAST2*, *NME5*, *OAZ3*, *PRKACG*, *PROK2*, *PTCH2*, *SERPINA5*, *SOX30*, *SPAG11B*, *STRBP*, *TESK1*, *TNP2*	6.26E-26 3.22E-02
Cell cycle	8	*APOE*, *CCNA1*, *CHEK1*, *DMC1*, *DUSP13*, *GNAS*, *H1FNT*, *PPAP2A*	9.09E-05 4.61E-02
Cellular assembly and organization	6	*ACHE*, *APOE*, *BCL2L10*, *CHEK1*, *SPAM1*, *TNP2*	1.90E-04 4.96E-02
Cellular movement	11	*AKAP3*, *APOE*, *GNAS*, *HMGCR*, *MAK*, *PPAP2A*, *PROK2*, *SERPINA5*, *SLC22A16*, *STRBP*, *TNP2*	5.64E-04 1.80E-02
Cell-to-cell signaling and interaction	9	*ACHE*, *ADAM28*, *APOE*, *CHEK1*, *GNAS*, *PPAP2A*, *SERPINA5*, *SPAM1*, *TNP2*	9.52E-04 4.96E-02

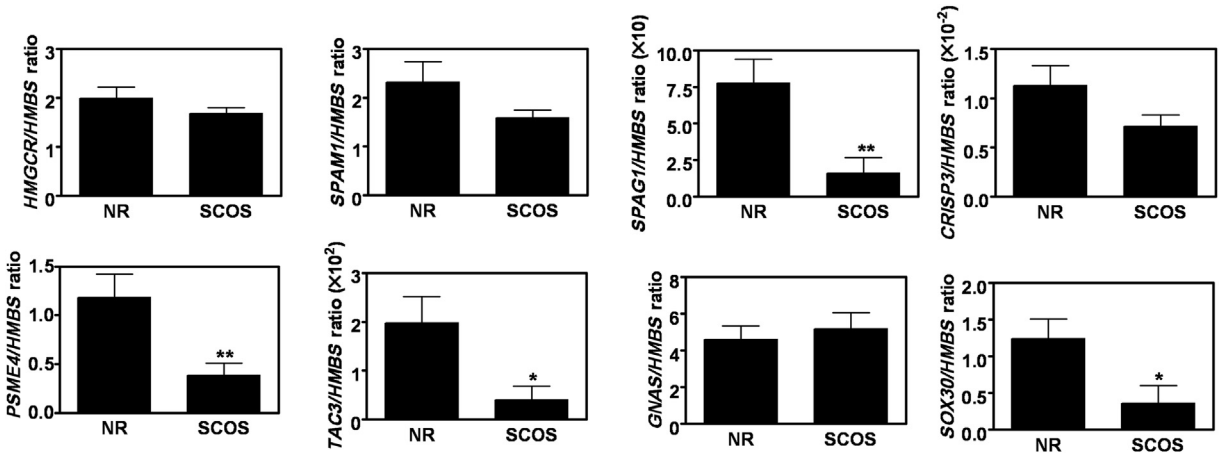

Figure 2. Quantitative real-time polymerase chain reaction analysis of microR-630 target expression. MessengerRNA transcript levels (compared with *HMBS*) for eight microR-630 target genes between the normal (NR) and Sertoli cell-only syndrome (SCOS) testes were determined using quantitative real-time polymerase chain reaction. Significant differences between the groups were found for *SPAG1*, *PSME4*, *TAC3*, and *SOX30*. * $p < 0.05$. ** $p < 0.01$. *** $p < 0.001$.

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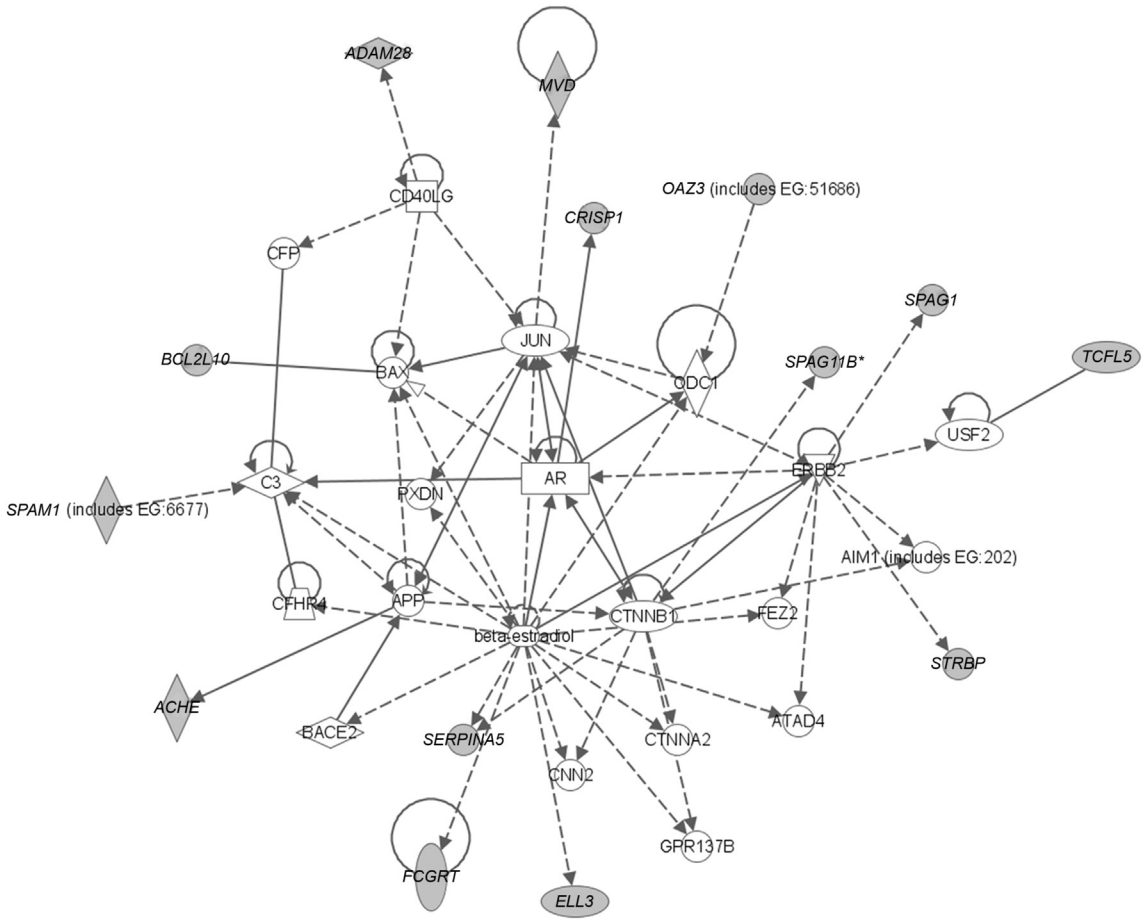

Figure 3. Network 1 identified by Ingenuity Pathway Analysis (IPA). This network consisting of 14 focus genes has an IPA score of 28. Androgen receptor is central in this network with proposed functions of cellular development and reproductive system development and function by IPA. The focus genes are shown by shading.

3.5. Molecular network analysis and canonical pathway analysis

To investigate whether these predicted target genes form networks of interactions, we uploaded these targets for IPA analysis. The results showed that these target genes were overrepresented in six IPA networks converging on cellular development, reproductive system development and function, cell-to-cell signaling and interaction, cell-mediated immune response, cellular growth and proliferation, amino acid metabolism, and post-translational modification. The top three networks with an IPA score > 20 are shown in Figures 3–5, respectively. For canonical pathway analysis, a total of 31 canonical pathways were identified, of which five were significantly ($p < 0.05$) associated with the data. Table 3 shows these five pathways and their proposed functions in spermatogenesis.

4. Discussion

Here we have presented the results of a genome-wide miRNA profiling that offers a comprehensive look at miRNAs expressed in human testes. Through microarray analysis, we identify 10 miRNAs that are differentially expressed between normal and SCOS testes. Three miRNAs are upregulated, while the other seven miRNAs are downregulated in SCOS patients. Using bioinformatics and a computational search, we predicted their target genes known to be implicated in spermatogenesis. Results from qRT-PCR generally correlated with microarray and computational analysis. Additionally, network and canonical pathway analysis suggested several molecular mechanisms regulated by these target genes. We believe that these data provide the basis for elucidating the biological effects of miRNAs in human spermatogenesis.

Although microarray analysis yields a genome-wide identification of differentially expressed miRNAs, changes in gene expression may vary markedly between individual patients. Moreover, it has been shown that a piece of testicular sample may not actually represent the histology of the entire testis due to its heterogeneous nature of spermatogenic activity.[27] In this study, testicular specimens of the same histology were pooled to reduce the interpersonal variations and to minimize the biases of testicular biopsy. Although it may take into account that pooling strategy can result in a loss of sensitivity in identifying important miRNAs that have small, but statistically significant, changes in miRNA expression, the results from our pooling strategy have the potential to be applied to most human patients.[7]

Our results demonstrated that miRNAs have different profiles in SCOS compared with normal spermatogenesis, which are informative for reflecting the roles of miRNAs in spermatogenesis. Those miRNAs whose expression is increased in SCOS may be considered as “sterility” miRNAs. These miRNAs are thought to induce spermatogenic failure by negatively inhibiting genes that control germ cell development or differentiation. By contrast, those miRNAs whose expression is decreased in SCOS may be considered as “fertility” miRNAs. These types of miRNAs are likely to promote spermatogenesis. According to literature, none of the 10 miRNAs presented here have ever been shown to be associated with gonadal development or spermatogenesis. This may simply reflect

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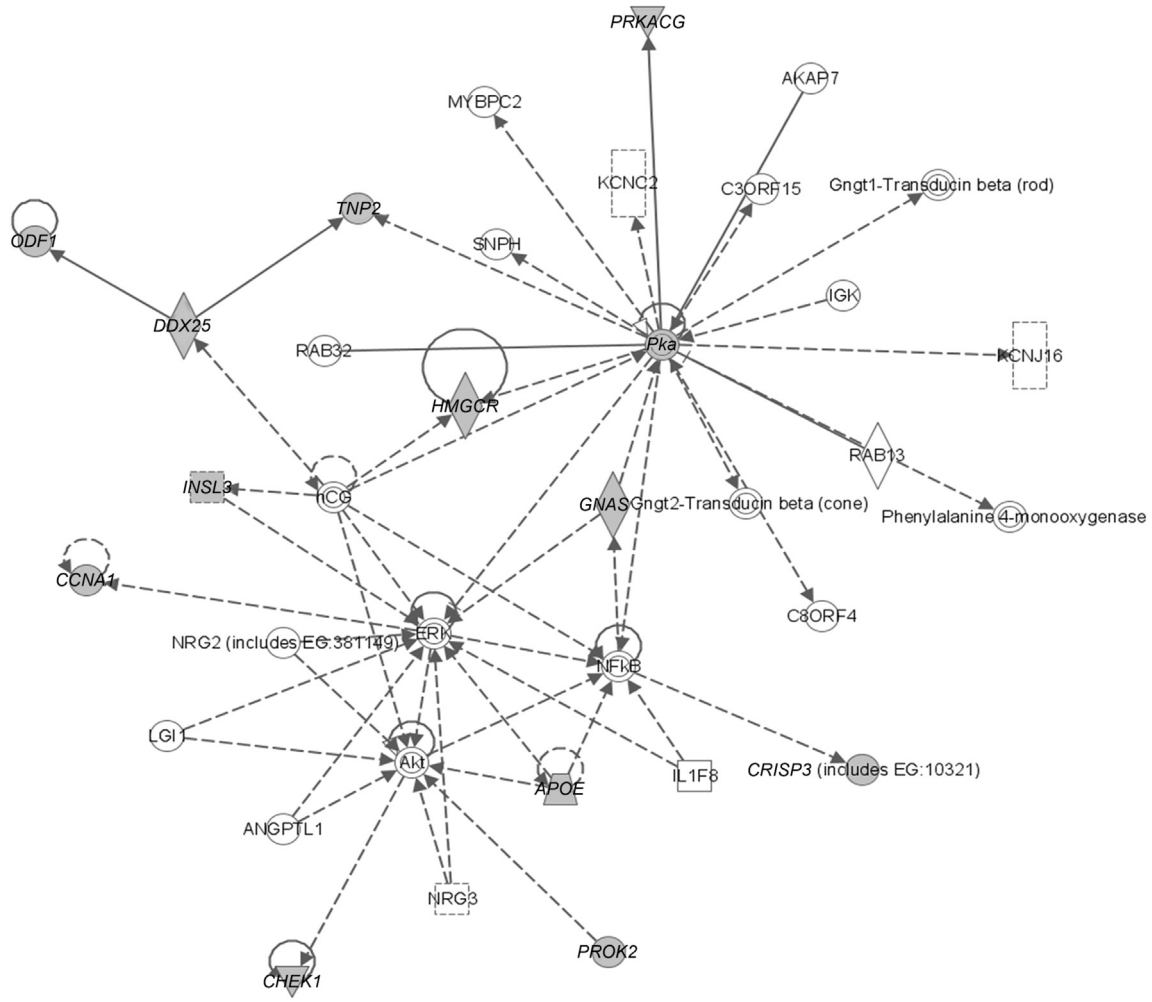

Figure 4. Network 2 identified by Ingenuity Pathway Analysis. This network consisting of 12 focus genes has an Ingenuity Pathway Analysis score of 25. *PKA* and *ERK* play important roles in the formation of this network. This network has proposed functions associated with reproductive system development and function, cellular development, and organ development. The focus genes are shown by shading.

the limited research of miRNA in this area. Thus, our data suggest the novel biological functions of these miRNAs in human spermatogenesis.

Identification of miRNA targets is essential to understand the biological functions of miRNAs. In this study, we predicted 51 spermatogenesis-related genes targeted from the 10 miRNAs. IPA analysis shows that individual genes were found in multiple categories of molecular and cellular functions related to cellular development, cell cycle regulation, cell-to-cell signaling and interaction, cellular movement, and cellular assembly and organization. Indeed, all of these functions are crucial for spermatogenesis. We then tested whether upregulation of miRNA is associated with the degradation of the target transcript. miR-630 was randomly selected, and the transcript levels of its target genes were determined. Of the eight target genes, *SPAG1*, *PSME4*, *TAC3*, and *SOX30* transcripts were significantly decreased in SCOS specimens, suggesting that miR-630 might induce transcript degradation of these genes. In contrast, transcript levels of the *HMGCR*, *SPAM1*, *CRISP3*, and *GNAS* genes are not different between normal and SCOS testes, suggesting that miR-630 might lead to the accumulation of target mRNAs in the P- or GW-bodies,[28,29] isolating mRNAs from translation. Thus, only significant changes in the protein level, but not in RNA level, are found.[30–32] However, given that each mRNA may be controlled by several miRNAs or transcriptional factors, it is also possible that miR-630 can bind the complementary binding sites within these target genes but play little role in controlling the gene expression. Therefore, our results raise the importance of experimental validation of miRNA/mRNA interaction in determining the effect of miRNA on target's expression.

In this study, functional analysis identified three high score networks containing target genes linking to SCOS. Network 1 primarily centers on the *androgen receptor* gene. It is well known that androgen is essential for fetal and pubertal gonadal development and that mutation/polymorphism of the *androgen receptor* gene can cause a wide spectrum of androgen insensitivity syndromes, from ambiguous genitalia to idiopathic male infertility.[33] Network 2 is identified around *PKA* and *ERK* genes. Cyclic AMP-PKA pathway is required for steroidogenic acute regulatory protein synthesis and steroidogenesis,[34] and *ERK* has recently been shown to regulate steroidogenic acute regulatory protein expression in conjunction with the *PKA* and *PKC* pathways,[35] suggesting that molecules involved in this network are essential for testicular steroidogenesis. Network 3 shows a lot of activity around transforming growth factor-β1 that is a multifunctional cytokine. A number of studies have suggested that transforming growth factor-β1 plays crucial roles in the testicular development during puberty, in regulation of spermatogenesis and steroidogenesis.[36,37] Consistent with the findings from network analysis, the canonical pathway analysis

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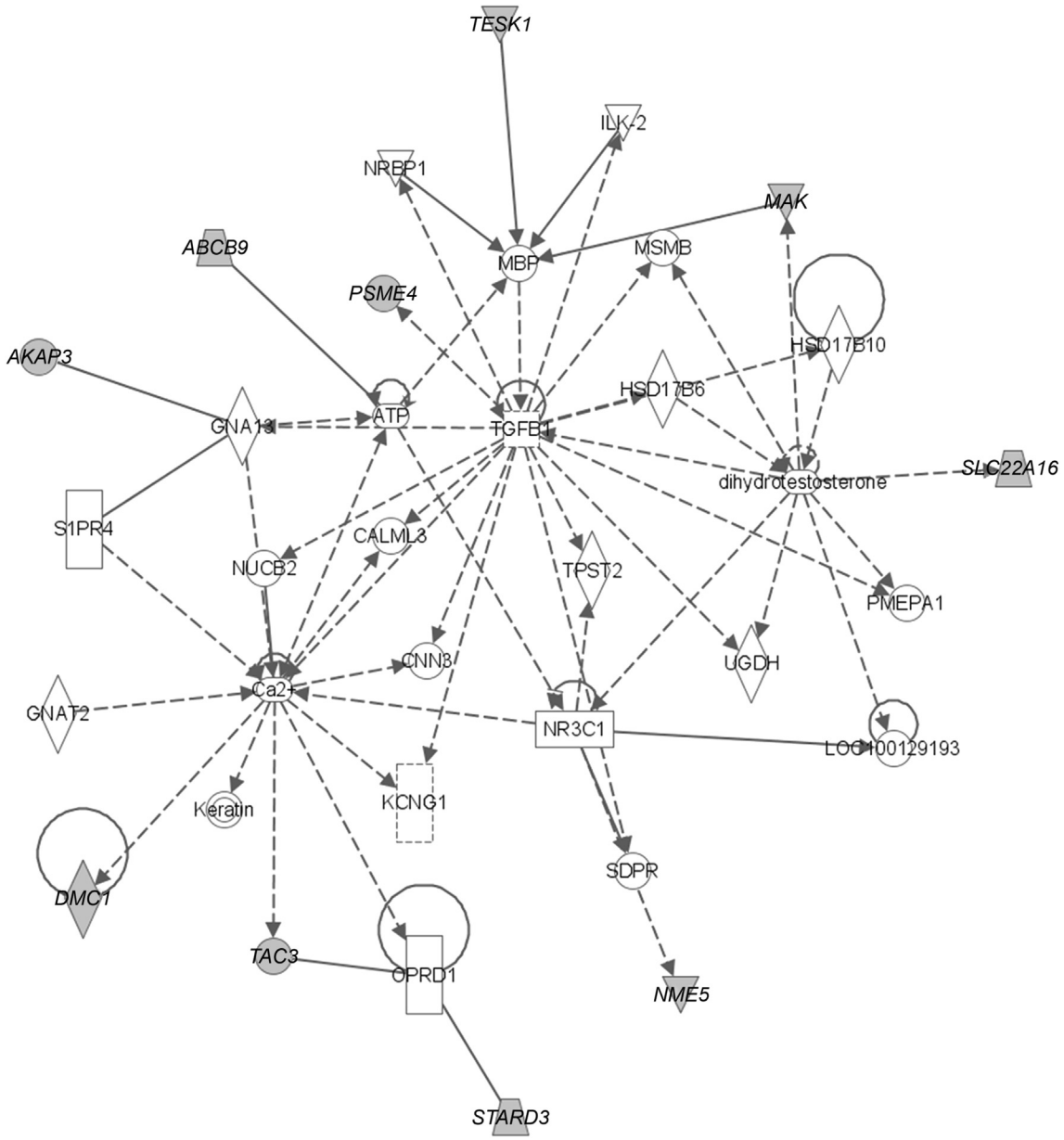

Figure 5. Network 3 identified by Ingenuity Pathway Analysis. This network consisting of 10 focus genes has an Ingenuity Pathway Analysis score of 20. This network shows a lot of activity around transforming growth factor-β1, suggesting roles in cell-to-cell signaling and interaction, cell-mediated immune response, cellular growth, and proliferation. The focus genes are shown by shading.

Table 3
Significant canonical pathways of target genes and their proposed roles in spermatogenesis.

Canonical pathway	*p*	Proposed role in spermatogenesis
Biosynthesis of steroid	3.64E-03	Steroidogenesis
Sonic-hedgehog signaling	4.56E-03	M-phase cell cycle progression, transcriptional regulation, cell proliferation
cAMP-mediated signaling	1.68E-02	Regulation of transcription, Steroidogenesis, apoptosis
LXR/RXR activation	2.51E-02	Cholesterol metabolism, inflammatory mediation
Aryl-hydrocarbon receptor signaling	4.8E-02	Cell cycle progression, cell proliferation, apoptosis

LXR = liver X receptor; RXR = retinoid X receptor.

revealed that steroid biosynthesis-related pathways and cell cycle-related pathways are the most significant signaling pathways modulated by these target genes. Thus, the data suggest that miRNAs might regulate diverse functions in multiple aspects of spermatogenesis and steroidogenesis. Dysregulation of these miRNA can result in spermatogenic failure that may ultimately lead to SCOS.

Our data provide the potential causative roles of deregulated miRNA processes in human spermatogenesis. A better understanding of these networks and pathways will bring us closer to an

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understanding of the molecular mechanisms underlying the causes of SCOS. We are currently using luciferase reporter assay, a gene-silencing technique, as well as rescue assay to validate specific miRNA targets. If the biological functions and the relevant signal pathways of miRNAs are extensively studied, we expect that the miRNA profiles may aid in the diagnosis of male infertility. Furthermore, antisense oligonucleotides complementary to miRNAs (antagomirs) or synthetic miRNAs (agomirs) can be generated. Given that therapeutic antagomir has been successfully used in nonhuman primates,[38] activation or silencing of miRNAs might become a therapeutic strategy for male infertility treatment in the future.

Conflicts of interest

All contributing authors declare no conflicts of interest.

Acknowledgments

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